This report is required by law (7 USC 2143). Failure to report according to the regulations can result in an order to cease and desist and to be subject to penalties as provided for in Section 2150.

See reverse side for additional information.

Interagency Report Control No 0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

1. REGISTRATION NO. 58-R-0003 **CUSTOMER NO.** 859

FORM APPROVED
OMB NO. 0579-0036

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF FLORIDA
P.O. BOX 115500
GAINESVILLE, FL 32611
(352) 392-9271

3. REPORTING FACILITY (List all locations where animals were housed or used in actual research, testing, teaching, or experimentation, or held for these purposes. Attach additional sheets if necessary.)

FACILITY LOCATIONS*(sites)*

See Attached Listing

NOV 2 2006

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use APHIS FORM 7023A.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS **(Cols. C + D + E)**
4. Dogs		39	88		127
5. Cats		56	340		396
6. Guinea Pigs		88			88
7. Hamsters		0			0
8. Rabbits		68	169	60	297
9. Non-Human Primates		8	110		118
10. Sheep		50	76		126
11. Pigs		44	70		114
12. Other Farm Animals					
Horses		166	132	20	318
13. Other Animals					
Chinchillas		4	18		22
Deer Mice		603	90		693
Singing Mice		96			96

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL

(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

(b)(6), (b)(7)c

DATE SIGNED 11-21-06

APHIS FORM 7023 (AUG 91) (Replaces VS FORM 18-23 (Oct 88), which is obsolete

PART 1 - HEADQUARTERS

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NOV 24 2006
BY:

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See reverse side for additional information.

Interagency Report Control No
0180-DOA-AN

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE

1. REGISTRATION NO. 58-R-0003

CUSTOMER NO. 859

FORM APPROVED
OMB NO. 0579-0036

CONTINUATION SHEET FOR ANNUAL REPORT OF RESEARCH FACILITY

(TYPE OR PRINT)

2. HEADQUARTERS RESEARCH FACILITY *(Name and Address, as registered with USDA, include Zip Code)*

UNIVERSITY OF FLORIDA
P.O. BOX 115500
GAINESVILLE, FL 32611
(352) 392-9271

REPORT OF ANIMALS USED BY OR UNDER CONTROL OF RESEARCH FACILITY *(Attach additional sheets if necessary or use this form.)*

A. Animals Covered By The Animal Welfare Regulations	B. Number of animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery but not yet used for such purposes.	C. Number of animals upon which teaching, research, experiments, or tests were conducted involving no pain, distress, or use of pain-relieving drugs.	D. Number of animals upon which experiments, teaching, research, surgery, or tests were conducted involving accompanying pain or distress to the animals and for which appropriate anesthetic, analgesic, or tranquilizing drugs were used.	E. Number of animals upon which teaching, experiments, research, surgery or tests were conducted involving accompanying pain or distress to the animals and for which the use of appropriate anesthetic,analgesic, or tranquilizing drugs would have adversely affected the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests. *(An explanation of the procedures producing pain or distress in these animals and the reasons such drugs were not used must be attached to this report)*	F. TOTAL NO. OF ANIMALS (Cols. C + D + E)
Cattle		25	4		29
Voles		125	65		190
Hyrax			4		4

ASSURANCE STATEMENTS

1) Professionally acceptable standards governing the care, treatment, and use of animals, including appropriate use of anesthetic, analgesic, and tranquilizing drugs, prior to, during, and following actual research, teaching, testing, surgery, or experimentation were followed by this research facility.

2) Each principal investigator has considered alternatives to painful procedures.

3) This facility is adhering to the standards and regulations under the Act, and it has required that exceptions to the standards and regulations be specified and explained by the principal investigator and approved by the Institutional Animal Care and Use Committee (IACUC). **A summary of all the exceptions is attached to this annual report.** In addition to identifying the IACUC-approved exceptions, this summary includes a brief explanation of the exceptions, as well as the species and number of animals affected.

4) The attending veterinarian for this research facility has appropriate authority to ensure the provision of adequate veterinary care and to oversee the adequacy of other aspects of animal care and use.

CERTIFICATION BY HEADQUARTERS RESEARCH FACILITY OFFICIAL
(Chief Executive Officer or Legally Responsible Institutional official)

I certify that the above is true, correct, and complete (7 U.S.C. Section 2143)

(b)(6), (b)(7)c

DATE SIGNED 11-21-06

APHIS FORM 7023A
(AUG 91)

(Replaces VS FORM 18-23 (Oct 88), which is obsolete

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PART 1 - HEADQUARTERS

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 40 of animals used in this study.

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: D968; Antiviral activity of lipid-conjugated nucleoside analogs against orthopoxviruses

   Please see attached for procedure information.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency____________________ CFR____________________

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4. Explain the procedure producing pain and/or distress, including reason(s) for species selected.

Rabbits infected with rabbitpox virus are a unique animal model for smallpox studies because the disease closely mimics the pathology of the human disease, and because rabbitpox virus can be spread between animals in the absence of mechanical or insect vectors. In addition, federal regulations require the use of 2 animal species when using animal data to establish efficacy for human drugs.

Procedures for restraint of the rabbits will be as follows:

Animals will be restrained in a rabbit restrainer (provided by ACS) or by using a clean towel with minimal restraint by an animal handler.

Procedure for Administration of Virus:

The rabbits will be restrained using a rabbit restrainer or with a towel and both hind flanks will be shaved. The rabbits will be gently restrained with a towel and then injected intradermally with 100ul of buffer and the virus injected intradermally in one or two sites using a 25 gauge needle in one or both hind flanks.

Procedure for microchip placement:

This procedure does not cause excessive pain to the animal as the chip is placed just under the skin. The benefits of placing the chips into the animals and having them experience a momentary needle stick is far less stress than the alternative which is a rectal temperature taken multiple times within a day. A sterile Implantable Programmable Temperature Transponder chip (Biomedic Data Systems, Inc.) will be injected subcutaneously at the nape of the neck (dorsal cervical region). The chip transmits data for animal identification and body temperature. The rabbits will be gently restrained with a towel. The skin over the nape of the neck will be gently tented away from the back muscles. The tented fur at the nape of the neck will be washed with ethanol. A 2.2 by 14 mm transponder chip will be inserted under the skin by injection via the sterile 14 gauge needle that houses the chip. The skin will be released and physically examined to ensure the chip is placed under the skin and that there is no damage to the skin.

Routine Physical Exam:

A routine physical exam (abdominal palpitation, auscultation of the heart and lungs, assessment of attitude and hydration, etc.) will be performed daily.

Collection of Blood (Survival):

Rabbits are minimally restrained. Approximately an area of 4 inches by 4 inches of the hind flank of the animal is shaved and the skin is cleaned with 70% ethanol. 1-2ml of blood is collected from the lateral saphenous or ear vein with a 25 gauge needle daily. Kidney parameters will not be tested as CDV has not been observed to be nephrotoxic at the dose that is proposed. There has been no nephrotoxocity observed in animals (rabbits as well as mice) given LIP-CDV.

Collection of Blood (Non-survival):

For collection of 10ml blood, 50mg/kg ketamine and 10mg/kg xylazine will be injected intramuscularly into the biceps femoris muscle or intraperitoneally. Blood will be collected by cardiac puncture using a sterile 22 gauge 1 ½ inch needle attached to a 12cc syringe. 100-150mg/kg body weight of pentobarbital will be injected into the heart after blood has been collected to induce euthanasia.

Necropsy and Tissue collection:

Upon euthanasia with pentobarbital and the confirmation of successful euthanasia, as determined by the absence of heart beat and respiration, a necropsy of the animal will be performed. Tissue samples from all the organs, as well as lesions on the dermal phase of the animal, will be collected for virus isolation and characterization.

Administration of Cidofovir:

Cidofovir will be administered in an ear vein dosed at 5-20 mg/kg.

Administration of LIP-CDV:

The oral LIP-CDV will be administered in a liquid form sweetened with sugar water at a dose of 5-20 mg/kg/day with the animals receiving a total volume of no more than 10ml at a given dosing event.

The intradermal administration of rabbitpox virus will produce both a primary lesion at the site of administration as well as systemic disease. (Bedson, H.S. and Duckworth, M.J. (1963). J.Pathol.Bacteriol. 85, 1-20.) The skin lesions are not treated. Death from the rabbitpox infection in the wild, and from experimental infection, is usually a direct result of respiratory failure, and occurs between days 8 to 14. The animals are monitored daily for the first eight days and body weights measured. After the first eight days, the animals are monitored three times between the hours of 8 am and 8 pm and once at night (approximately every 6 hours) up to day 14. A set of criteria for scoring the level of disease in the animals has been created and is attached. Although these animals will develop significant respiratory distress, death is not a desired outcome and animals will be euthanized when evidence of respiratory distress is present. A physical exam (including respiratory and heart rates, core temperature, and examination of all major organ systems) is performed daily, and the size of the primary lesions are measured. Any animal with frank lung sounds, markedly reduced respiratory rate, open mouth breathing, temperature equal to or exceeding 106°F, anorexia, dehydration, and/or morbidity will be euthanized according to the AVMA Panel on Euthanasia. The symptoms of the disease are not predictable enough to end the studies early. Some animals may develop increased lung sounds that clear within 24-48 hours and the animal make a full recovery. We also have an idea of how the drugs will influence the disease progression, but we must follow the disease in the animals until the appropriate endpoint, which is respiratory distress.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results.

Rabbitpox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. It is critical to be able to identify any potential drug related toxicities during the course of these experiments. Analgesics are not indicated in these studies because of their affects on the inflammatory and immune responses that could possibly hinder the typical clinical observations previously documented in rabbits infected with RPV. It is critical that the rabbitpox viral disease be observed unhindered.

Column E Explanation

This form is intended as an aid to completing the Column E explanation. It is not an official form and its use is voluntary. Names, addresses, protocols, veterinary care programs, and the like, are not required as part of an explanation. A Column E explanation must be written so as to be understood by lay persons as well as scientists.

1. Registration Number: 58-R-0003

2. Number 20 of animals used in this study.

3. Species (common name) Rabbits of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: Gene regulation of mammalian DNA viruses: Antiviral activity of Imantinib (Gleevec) against Orthopoxviruses
   Procedure: Please see attached.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency________________ CFR________________

E520; *Gene regulation of mammalian DNA viruses: Antiviral activity of Imantinib (Gleevec) against Orthopoxviruses*

4. Explain the procedure producing pain/or distress.

Concerns over smallpox, eradicated in 1980, have risen dramatically in recent years because of the potential for variola virus to be used as a bioweapon. Vaccination, considered by many as the first line of defense in the case of an intentional release, is contraindicated in a significant percentage of the US population, making development of antiviral agents effective against variola virus critical to preventing widespread mortality in the event of a bioterrorist attack. Prior to conducting human trials with potential anti-poxvirus compounds, the efficacy of selected drug candidates must be demonstrated in at least 2 animal models.

This is an application to conduct efficacy studies of Imatinib, or Gleevec, in orthopoxvirus infected rabbits. Imatinib is an FDA approved medication currently used to treat chronic myelogenous leukemia in humans and has been shown to interfere with the release and spread of the Orthopoxvirus, vaccinia virus, within cell culture. It has also proven effective at protecting mice from lethal vaccinia virus challenge. We intend to determine the drug's effectiveness in protecting New Zealand white rabbits from a lethal challenge with the Orthopoxvirus, rabbitpox virus, as a model system for characterizing antiviral activity. The goal of these experiments is to 1) determine if orally administered Imatinib is effective in preventing and/or treating orthopoxvirus infection in rabbits, and 2) identify the most effective dose and dosing regimen.

These experiments will consist of treating the animals by orally administering Imatinib at various doses, and infecting animals with a lethal dose of rabbitpox virus by intradermal injection. Rabbits will be closely monitored for signs of disease, and euthanized if they develop severe illness (as indicated by decreased respiratory rates, dyspnea, open-mouth breathing, severe lung sounds, temperature equal to or exceeding 106°F, anorexia (not to exceed four days), dehydration, and/or morbidity).

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

Rabbitpox is a lethal infection in rabbits. Analgesics are not indicated in these studies because their affects would make it difficult to identify any adverse effects caused by drug treatment. Analgesics can have effects on respiration rate (a major criterion of euthanasia), temperature, heart rate and the general demeanor and behavior of the animal (attitude and posture). For example, personal communications with another RPV researcher at USAMRIID indicated they did not observe a temperature spike in infected animals, as they used analgesics every other day before drawing blood, and this suppressed the normally observed fever. It is critical to be able to identify any potential drug related toxicities during the course of these experiments.

Column E Explanation

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1. Registration Number: 58-R-0003

2. Number 20 of animals used in this study.

3. Species (common name) Horses of animals used in the study.

4. Explain the procedure producing pain and/or distress.

   Title: D163; West Nile Virus/Live Yellow Fever Vector Vaccination: Immunogenicity Testing
   Procedure: Please see attached.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)

   Please see attached.

6. What, if any, federal regulations require this procedure? Cite the agency, the code of Federal Regulations (CFR) title number and the specific section number (e.g., APHIS, 9 CFR 113.102):

   Agency__________ CFR__________

D163; *West Nile Virus/Live Yellow Fever Virus Vector Vaccination: Immunogenicity Testing*

4. Explain the procedure producing pain/or distress.
The mosquito-borne flavivirus, West Nile (WN) virus, is a newly emerging disease in the United States. This flavivirus was an old world virus of Europe, Asia, Africa and the South Pacific until 1999, when it was discovered as the cause of encephalitis and death in birds, humans, and horses of the New York City area. During 2002, over 2000 people were confirmed with WNV meningoencephalitis, 14,717 horses were confirmed for West Nile Virus encephalomyelitis, and over 120,000 dead bird reports were investigated. A private biotechnical company, in collaboration with the NIH, has developed vaccine technology that relies on creating recombinant flaviviruses. The backbone of yellow fever virus (YF17D) has been primarily used. Two proteins important for protection, the E protein and prM, (those important for neutralization) have been cloned into this YF backbone. As a result both the YF part of the virus has decreased its ability to cause disease. There is a profound need to develop safe, modified live vaccines that provide long-lasting immunity to West Nile virus in both horses and humans. Presently, a vaccine for the sister virus, Japanese Encephalitis Virus is in stage II trials for human licensure. The one objective of this study is to demonstrate the immunogenicity of a vaccine to horses. The hypothesis of this grant is that ChimeriVax® can be safely used in horses to protect against West Nile virus. Horses will receive the vaccine and infected with live West Nile and monitored for protection against clinical disease.

5. Provide scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that pain and/or distress relief would interfere with test results. (For Federally mandated testing, see Item 6 below)
Horses will be observed daily every 6 hours which includes the night. 24 hours per day while stalled at the Progress Center. Once per day a full physical and neurological examination will be performed. The study has budget a full time horse manager. This person will be at the Progress Center throughout the day so technically, during his 8 hour shift, and the animals will be observed several times.
It is expected that ALL of the nonvaccinated horses will develop clinical signs consistent with West Nile virus infection.
With this model, horses develop clinical signs within 7-9 days post-inoculation. The initial signs are fever, depression, anorexia, muscle and skin fasciculations. Within 2-4 days horse may develop spinal weakness and ataxia. Thirty-five percent of horses become recumbent after the onset of spinal weakness and ataxia. Horses will be euthanized within 24 hours of systemic clinical signs and fasciculations, it is not expect that any will become significantly neurologic or recumbent.
Thus far West Nile virus has been reported in at least 15,500 U.S. horses since its encroachment in 1999. Under experimental conditions, researchers have been unable to induce clinical West Nile virus. In 2001 a killed vaccine was marketed under a conditional license and this became fully licensed March 2003. As such NO OTHER VACCINE can be conditionally licensed for West Nile virus. Standard infection methods employed during the licensing of the killed product failed to cause clinical disease. Thus, this vaccine is only labeled for prevention of viremia. Although horses

are highly clinically susceptible to infection, they develop low viremia. Thus the requirements for the vaccine already marketed fall short in terms of clinical efficacy. Vaccinated horses have developed fulminate West Nile virus and it appears the marketed vaccine is of short duration. Intervet is attempting to label this vaccine for prevention of disease. In order to demonstrate efficacy, clinical disease must be induced and the intrathecal method appears successful thus far in induction of disease.